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SUB GINGIVAL COMPLEX MICROBIAL BIOFILMS AND THEIR ERADICATION

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Abstract

Aim of study: The study was conducted to discuss and explain the ability to use a new methods for removal and even prevention of sub gingival biofilm.
Biofilm is a three dimensional structure microbial community that first noted in the oral cavity as plaque on the crown of teeth which is called later supra gingival plaque to play a role in caries development . Un eliminated supra gingival plaque grow sub gingivaly to form more protected biofilm found in the gingival sulcus that is responsible for the conversion of physiological gingival sulcus (0-3 mm) to pathological pocket > 3mm which play a role in the pathogenesis of different periodontal infections. Sub-gingival microbiota include mainly anaerobic proteolytic gram negative rods, these microorganisms considered as late colonizer which required the early colonizer to adhere primarily producing specific receptors, reducing the oxidation reduction potential (Eh), cooperate metabolically within the biofilm. The study was based on a review submitted by: Cortés, et.al. (2011) titled " Biofilm formation, control and novel strategies for eradication. The study took place during the period (Oct. 2022 - Feb. 2023) The challenges which faces oral microbiologist were characterizing the composition of sub gingival bacterial biofilm since most of them are un cultivable hence new molecular technique were facilitate these difficulties which in turn open horizons for eradication of these resistant microbial biofilm bacterial cells for novel treatment strategies and to prevent recurrence of periodontal infections.

Keywords: Sub Gingival, Periodontopathogens, Erradication.

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Introduction

Biofilm formed on biomedical devices, oral prosthodontic, orthodontic appliances and dental implants considered an important issue to be solved since its lead to chronic recurrent infections that resist antimicrobial and antiseptic agents[1].

1.1. Biofilm definition and characteristics

Biofilms are the aggregation of mono or poly microbial species cells , which are adhered to surface irreversibly, i.e. difficult be dislodged even by sonication [2]. Biofilm cells are attached to a biotic or abiotic surface. A close proximity of the microbial cells embedded within biofilm community enhances gene transformation. Once the cells become adherent they will undergo phenotypic, genotypic changes they have altered gene expression pattern and low metabolic growth rate render them resistant to traditional antimicrobial agents, all these biofilm feature encountered by oral microbiologist and periodontist trail looking for anti biofilm strategies to eliminate remaining resistant and adherent biofilm cells causing recurrent periodontal infection even after scaling, root planning and antiseptic mouth wash treatment [3].

1.2. The compositions of biofilm

Biofilm consist mainly of micro-colonies which are rod-like or mushroom-shaped non randomly arranged within the glycocalyx. Each biofilm have accustomed architecture Figure (1).These microcolonies are either of the same or different species. Water constitutes 91% of biofilm volume through which the microorganisms are seeded and acts as a vehicle for nutrients through the biofim channels, while the rest 9% is consist of microorganisms in the form of micro- colonies and extracellular matrix, which consist of exo-polysaccharides(EPS),proteins, DNA, RNA and ions depending on the microorganisms producing them and environmental conditions [4].The outer layer of the biofilm is loosely attached and responsible for biofilm dispersion. The bottom layers of the biofilms, is bound on the surface together with the extra cellular matrix [6] The study of biofilm components specially the type of extra-cellular matrix play a role in the selection of the treatment strategies needed for biofilom degradation[5].

Figure (1) Mono spp. Biofilm architecture by *A. actinomycetemcomitans* from subgingival plaque of patient with aggressive periodontitis showing 3D structure (lab. of microbiology at Mosul university College of dentistry Dental basic sciences)

1.3. Biofilm development:

Oral biofilm formation is dynamic process dipected as a developmental cycle. The cycle is consist of four steps (attachment, proliferation, maturation and dispersion) all these steps are influenced by biological, physical, and environmental factors. Initial attachment (reversible) depend on short range adhesion(nearness) of microbial cells to the surface, its provide a chance for irreversible attachment to take place by mean of electronic interaction. Proliferation involve synthesis of different types of extracellular matrix depend on embedded bioflm cells [6]. Biofilm is maturated by the of chemical signals known as quorum sensing molecules to increase the population of the cells within the biofilm to reach threshold level that alter gene expression pattern. Water channels are formed to deliver water, nutrients and oxygen to the deep layer and excretes west products. Dispersion of the biofilm is mandatory allowing cells to spread seeking for nutrients and appropriates conditions [7].

2.1 . Periodontal diseases a recurrent biofilm infection:

Periodontal disease is classified mainly to gingivitis and periodontitits, its an immune pathogenic biofilm infection, according to biofilm existence recent classification of gingivitis to biofilm induced and non biofilm induced gingivitis There has been a strong association between biofilom and gingivitis which is prevalent in humans, affecting more than 90% of the adult population . compared to periodontitis which are affecting about 20% of the adult individuals and less abundant despite the existence of plaque in most cases [8]. Periodontal disease is caused by the effect of virulence factors produced by bacteria in the sub-gingival biofilm that play a role in the initiation and progression of the disease due to their interactionwith the immune response[9].

2.2 . Sub gingival periodontal pathogens

Bacteria isolated from sub-gigival plaque are divided into complexes according to their virulence factors and pathogenicity. These complexes are interact together to create climax community, Socransky and Haffagee [10]. classified sub-gingival microbial complex into six groups with specific color code to facilitate their study Table (1)

Bacterial species	Color coded complex
Actinomyces, Veilonella	purple
Streptococcus intermedius, mitis, sanguis,gordonii	Yellow
Capnocytophaga, Ecorrodens	Green
Campylobacter rectus, F.nucleatum, P.micros, P intermedia	Orange
T. forsythia, P.gigivalis, T.denticola	Red
A.actinomycetemcomitans, Selenomonas	Not grouped

Table (1) Sokranskys color coded complex of sub-gingival microbial complexes

Microorganisms in the yellow, green, purple, and blue complexes are considered as primary colonizer associated with gingival health mainly, while microorganisms in the orange group are secondary colonizers that acts as bridge for attachment of the tertiary colonizers which are aggressive periodontal pathogens that causes loss of attachment and deep periodontal pocket as a result of connective tissues and bone loss due to its proteolytic activity [11].

3.1. Biofilm eradication

Although biofilm eradication particularly oral biofilm is very challenge, but its always depend on simple conventional strategies by mechanical removal like scaling, polishing, brushing and root planning, removing plaque accumulated in the sub-gingival area, reduce disease progression and bacterial recolonization on the tooth surface[12]. This strategy has caused a reduction in the number of periodontal pathogens, it was reported in study were the bacterial load of *P. gingivalis* and *T. denticola* was decreased following mechanical treatment. More over , this treatment has improve clinical singes like reduction of pocket depth[13]. However, this method is drawn back or limited

because of the technical difficulty in biofilms removal located in very deep pockets,

root bifurcations. In addition relapse is very high since remaining bacterial cells embedded within the sub-gingival biofilm are highly resistant causes recurrent infection and some periodontopathogens such as *P. gingivalis* and *A. actinomycetemcomitans* can penetrate the tissue[14]. Recently a new strategies have been studied to prevent biofilm formation or remove biofilm .

3.2. Novel treatment strategies for biofilm prevention and removal

Eradication of persistent and resistant biofilm adherent cells was the border line between novel and traditional treatment of periodontal diseases. Biofilm can be eradicated during any step of biofilm development that are formed on biomedical devise like materials used for fabrication of oral devises and dental implants :

3.2.1. Inhibition of biofilm attachment either by : alteration of physical properties of material like surface roughness, charge and hydrophobacity. Surfaces modification can reduce attachment to restrict the microbial adherence e.g. electropolishing of stainless-steel. Many measures have been used to evaluate the materials surface roughness like(Ra value) which is the arithmetical mean deviation of the profile , the maximum peak to valley height in the sample length(Rt value) and the average maximum profiler height (Rz values). Recently scanning electron microscopy (SEM) produce a three-dimensional view of the surface topography and atomic force microscopy (AFM) is used to determine the three-dimensional topographical parameters in the nanometer scale [15].To overcome microbial adherence on biomedical device, surface can be coated by that are either antimicrobial agents, nonbiofuling materials, nanoparticles natural extracts with antimicrobial and antibiofilm properties ,polymethacrylate derivate with cationic side chain that becomes zwitterionic upon conversion of a terminal ester to carboxylate]. In this technology 99.9% of the attached bacteria were eliminated after 1 hour of exposure to the initially prepared coating [16]. Titanium Coated surface demonstrated that roughness on the nanometer scale-and not micrometer scale- increases the bacterial adherence, the study concluded that topography is the most important factor that influence bacterial adhesion [17]. The chitosan nanoparticles (CNPs), have good antimicrobial and anti adherent properties, owing to a higher surface charge density, These nanoparticles are able to interact with the negative charge surface of bacterial cells, leading to cell death. low molecular weights chitosans , exhibit high antimicrobial effect towards *S. mutans* bioflm [18,19]. Adherence of *Porphyromonas gingivalis* and *S. mutans* on the dental implants are the essential causative factor of

implant failure and peri-implantitis. Therefore titanium dental implants can be coated with Te Ag- conjugated CNPs to inhibit the biofilm formation of *Porphyromonas gingivalis* and *S. mutans* [20]. The second method to inhibit biofilm attachment is by altering the chemical properties by incorporating antibiotics, nanoparticles, natural extracts with antimicrobial properties into the resin and adhesive systems .Quaternary ammonium salts (QAS). They are polycations their positive charge is bind to the negative charge of bacterial cell membrane, causes cell lyses to act as broad-spectrum antimicrobial agent with low toxicity therefore added into the resin and adhesive system. At first theywere added in mouthwash to reduce oral bioflm in the 1970s and later added into composite restorative materials and resin in the 1990s [21]. Different types of QAS like quaternary ammonium dimethacrylate (QADM), that possess active groups on both ends of a dimethacrylate to be added to resin without compromising its mechanical properties . Another QAS is 12-methacryloyloxydodecyl- pyridinium bromide (MDPB), introduced by Imazato et al., exhibit potent antibacterial and antibioflm properties against E. faecalis, F. nucleatum, S. mutans, and Prevotella nigrescens[22,23]

3.2.2. Inhibition of biofilm maturation and growth by: interfering with quorum sensing molecules, Xavier and Bassler described intercellular communication which is depending on an autoinducing mechanism of chemical signals that differs between Gram-negative and

Gram-positive bacteriaThese signaling molecules have been broadly classified into three major classes: (1) N-acyl homoserine lactones (AHLs), produced by Gram-negative bacteria (2) autoinducing peptides, induced by Gram-positive bacteria (3) AI-2 maintain communication between Gram-positive and Gram-negative bacteria (interspecies communication). QS increase population density to critical level to control gene expression [24].Distruption of this communication system can be carried by(1) inhibition of AIs synthesis(2) Blocking AIs receptor(3)Degradation of AIs molecules[25]. Several plant extracts possess QS inhibition activity. Plant-derived compounds are mostly secondary metabolites, most of these active ingredients are mostly secondary metabolites possessing antimicrobial and anti biofilm properties particularly anti quorum sensing, include quinones, saponins, FLs, tannins phenolics, phenolic acids, , coumarins, terpenoids, and alkaloids [26]. Benthic marine macroalga Delisea pulchra produce halogenated furanones which act as anti-QS compounds. They were competitively bind to the LuxR type proteins to inhibit the QS.[25,29].Extracts of garlic and edible fruits, exert anti-biofilm properties against different pathogens.[30. Streptococcus mutans antibiofilm effect of the natural compounds, embelin and piperine was reported by Dwivedi and Singh 2016 analyzed by microtiter plate method. They found that found that minimum biofilm inhibitory concentration of piperine was

0.0407 ± 0.03mg/mL,,while for embelin was 0.0620 ± 0.03 mg/mL [27]. A study investigated the effect anti biofilm and anti quorum sensing effects of alcoholic *glacyrriza glabara* extracts (50 µl of 1mg /ml) for 2 hrs on 24hrs biofilm of *A.actinomycetemcomitans* a strong periodontal pathogen isolated from sub gingival plaque samples of deep pockets evaluated by SEM [28].

3.2.3. Removal of mature biofilm by enzymatic degradation of extracellular matrix of biofilm. 2- (4-methoxyphenyl)- N-(3-{[2-(4-methoxyphenyl) ethyl] imino}-1,4-dihydro-2-quinoxalinylidene) ethanamine can inhibit the bioflm formation by S. mutans and S. sanguinis and promote the removal of mature bioflm of both. More importantly, this molecule have good anticaries activity by signifcantly decreasing the incidence and severity of smooth surface caries in vivo [29] (2500 µg/ml) lysozyme reported a statistically signifcant bioflm degrading effect of 10%. Both the test and the control solutions were incubated for 15 min at 36 °C on bioflms, and loosened bioflm mass was eliminated by shear stress with a vortex . Bioflms stained with (tetrazolium dye),were analyzed by fluorescent microscope [30]. Detergents and surfactants can efficiently eliminate mature bioflms [31]. Specially those derived from coconut oil and dimethylaminopropylamine, were greatly incorporated in cosmetic products (especially in washing-up liquids) with an acceptably low irritant potential [32].

Conclusions:

Since most of sub-gingival microorganisms embedded within biofilom are resistant to antibiotics and live in well characterized communities so their eradication will be challenged the new strategies were aim to prevent biofilm before it will become completely mature, this mainly may lead to reduce the recurrent periodontal diseases after traditional clinical treatment.

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